

has been successfully performed in a patient with HIV/AIDS, thereby representing a novel time course for desensitization. Our starting dose was higher than the 0.02-mg starting dose administered to a non-HIV patient during fluconazole desensitization; however, our initial dose was equal to or less than other protocols involving HIV-infected patients.<sup>1,5,6</sup> Given this patient's HIV status, we used a cautious desensitization approach (eg, dose escalation every 6 hours) because of the evidence that HIV-infected individuals may experience possibly more severe drug-induced reactions, which may result from enhanced T<sub>H</sub>2-type responses in the setting of HIV.<sup>7,8</sup>

Within 2 days, the patient was able to tolerate the full, therapeutic dose of 200 mg twice per day of oral fluconazole. This protocol was completed without any adverse reactions. Premedication with diphenhydramine, 25 mg, and famotidine, 20 mg, was administered 30 minutes before starting desensitization. Diphenhydramine, 25 mg, was then given every 8 hours during the protocol, and famotidine was given every 12 hours. The patient was not taking systemic steroids before or during the desensitization. This premedication regimen was chosen because the patient was determined to have a high likelihood of drug-induced allergic reactions.

This case demonstrates that a more rapid oral fluconazole desensitization may be possible in HIV-infected patients with a history of hypersensitivity to this medication. Further studies with more patients are needed to better characterize rapid desensitization protocols in patients with HIV/AIDS with fluconazole hypersensitivity.

SUNIT JARIWALA, MD\*

NATALIA VERNON, MD\*

GABRIELE DE VOS, MD\*

\*Allergy/Immunology Division  
Albert Einstein College of Medicine  
Bronx, New York  
nvernon@montefiore.org

1. Craig TJ. Desensitization for fluconazole hypersensitivity. *J Allergy Clin Immunol*. 1996;98:845–846.
2. Neuhaus G, Pavic N, Pletscher M. Anaphylactic reaction after oral fluconazole. *BMJ*. 1991;302:1341.
3. Abbott M, Hughes DL, Patel R, Kinghorn GR. Angio-edema after fluconazole. *Lancet*. 1991;338:633.
4. Muñoz P, Moreno S, Berenguer J, Bernaldo de Quirós JC, Bouza E. Fluconazole-related hepatotoxicity in patients with acquired immunodeficiency syndrome. *Arch Intern Med*. 1991;151:1020–1021.
5. Randolph C, Kaplan C, Fraser B. Rapid desensitization to fluconazole (Diflucan). *Ann Allergy Asthma Immunol*. 2008;100:616–617.
6. Takahashi T, Hitani A, Yamada H, et al. Desensitization to fluconazole in an AIDS patient. *Ann Pharmacother*. 2001;35:642–643.
7. Fodeman J, Jariwala S, Hudes G, Jerschow E, Rosenstreich D. Subcutaneous allergen immunotherapy in 3 patients with HIV. *Ann Allergy Asthma Immunol*. 2010;105:320–321.
8. Hussain H, Beall G, Sanwo M. Drug reactions in HIV/AIDS. *West J Med*. 1997; 167:344.

## IDENTIFICATION OF MOLD ON SEASONAL INDOOR CONIFEROUS TREES

Epidemic peaks of respiratory illnesses in all age groups are observed around December 25, specifically 1 week before and 1 week after for school-aged children and adults, respectively,<sup>1</sup> often raising

suspicion that a live, indoor, coniferous Christmas tree may be playing a role. The sole 1970 investigation<sup>2</sup> of Christmas tree allergy concluded that aerosolized ragweed, grass, and tree pollen on the tree bark or oleoresin were likely causes. In 2007, levels of airborne mold spores in an apartment increased from 800 spores per cubic meter before the introduction of a live conifer to 5,000 spores per cubic meter after its presence of 14 days, but no spore identification was made.<sup>3</sup> Because allergy testing can identify a patient's mold sensitivity, it would be useful to know what molds may be found on conifers used as Christmas trees.

During December 2007, faculty and staff of the Department of Pediatrics at Upstate Medical University, Syracuse, New York, were solicited to provide clippings of their home Christmas tree that had been placed in unused commercial zip-lock bags; 28 samples were submitted to the Clinical Microbiology Laboratory at University Hospital. A 1-in piece of each sample, including needles and stem, was placed into a sterile, plastic screw-cap tube containing 1.0 mL of sterile water. The sample was vortexed for 30 seconds, and 0.1 mL of the water was removed and plated onto each of the following media: Sabouraud dextrose agar (Emmon's modification), inhibitory mold agar, and brain heart infusion agar with sheep blood, gentamicin, chloramphenicol, and cycloheximide (Remel, Lenexa, Kansas). Cultures were incubated at 30°C in ambient air for 4 weeks. Phenotypic identification of molds was made by observance of macroscopic and microscopic characteristics using tape preparations stained with lactophenol cotton blue. Molds that could not be identified phenotypically were subcultured on Sabouraud dextrose agar (Emmon's modification) and incubated at 30°C in ambient air until visible growth was seen.

Nucleic acid was extracted using PrepMan Ultra Sample Preparation Reagent (Applied Biosystems, Foster City, California) according to the manufacturer's instructions. When visible growth was observed, 1 to 2 mm was removed with a sterile applicator stick and placed in 100 µL of PrepMan reagent, 50 µL of the supernatant was stored at –20°C until polymerase chain reaction (PCR) was performed, and 2.5 µL of supernatant was used as template for each amplification reaction.

Real-time PCR was performed targeting the internal transcribed spacer (ITS) region, which is present in all fungi.<sup>4,7</sup> Forward and reverse primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used, which encompass ITS1, 5.8S ribosomal DNA, and ITS2. Each 25-µL reaction contained 2.5 µL of prepared template, 3 µL of magnesium chloride (4 mM), 2.5 µL of Light Cycler Fast Start DNA Master SYBR Green I (Roche Diagnostics, Indianapolis, Indiana), and 1.25 µL of each primer (0.5 µM). Thermal cycling (Smart Cycler II, Cepheid, Sunnyvale, California) parameters were 95°C for 10 minutes, followed by 50 cycles of 95°C for 5 seconds, 60°C for 20 seconds, and 76°C for 30 seconds with a final extension at 72°C for 2 minutes. The PCR products were prepared using the DNA Clean and Concentrator-5 kit (Zymo Research, Orange, California). Cycle sequencing was performed using BIG DYE Version 3.1 (Applied Biosystems), and the products were separated on an ABI 3100 Genetic Analyzer (Applied Biosystems).

The BLAST function of the National Center for Biotechnology Information database was used to generate sequence alignments. Only entries showing 100% query coverage were considered. Species identification was acceptable when matches were 99.0% or greater with 0.9% or greater difference among species. Genus identification was limited if matches were 97% to 98.9% or if less than 0.9% differences were observed among species with a 99.0% or greater match.

Fifty-three individual molds were cultured from 26 specimens; 23 were identified by phenotypic methods, and 24 required ITS se-

**Disclosures:** Authors have nothing to disclose.

**Funding Sources:** This study was supported in part by the Children's Environmental Health Centers of New York.

© 2011 American College of Allergy, Asthma & Immunology.

Published by Elsevier Inc. All rights reserved.

doi:10.1016/j.anai.2011.03.003

Table 1. Identification of Mold Species From Conifer Specimens

Mold <sup>a</sup>	No. of isolates	No. identified phenotypically	No. identified by ITS sequencing
<i>Aspergillus</i> sp	10	4	6
<i>Penicillium</i> sp	9	5	4
<i>Cladosporium</i> sp	8	7	1
<i>Alternaria</i> sp	7	7	0
<i>Microdiploia</i> sp	4	0	4
<i>Pseudocercospora</i> <i>fraxini</i> ( <i>Mycosphaerella</i> sp)	2	0	2
<i>Hormonema</i> <i>dematioides</i> ( <i>Sydowia polyspora</i> )	2	0	2
<i>Nigrospora</i> sp	2	0	2
<i>Trichoderma</i> sp ( <i>Hypocrea</i> sp)	1	0	1
<i>Stachybotrys</i> sp	1	0	1
<i>Leptosphaerulina</i> sp	1	0	1
Unidentified	5	0	0
Lost	1	NA	NA
<b>Total</b>	<b>53</b>	<b>23</b>	<b>24</b>

Abbreviations: ITS, internal transcribed spacer; NA, not applicable.

<sup>a</sup> Names in parentheses are associated teleomorphs that demonstrated a close match in GenBank.

quence analysis (Table 1). Five nonsporulating molds did not amplify with the ITS primers and remained unidentified. One mold isolate was lost. Most isolates (34) were potential allergens: *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria*. Of the 26 samples yielding mold isolates, the number of different isolates varied from 1 to 6. The aforementioned 4 molds were found in individual samples as follows: *Alternaria*, 7; *Aspergillus*, 6; *Cladosporium*, 7; and *Penicillium*, 7. The remaining isolates were environmental colonizers or plant pathogens, with rare or no association with human disease.

The purpose of this study was to survey the variety of molds that could be isolated from live Christmas trees that resided in homes during the Christmas season. The use of DNA sequence analysis allowed identification of molds that could not be identified phenotypically. Most molds that were identified are potential allergens and have been shown to increase the risk of wheeze, persistent cough, and allergic sensitization in infants.<sup>8</sup> It is possible that molds were present in homes before the entrance of the conifers. Further studies seem warranted.

LAWRENCE E. KURLANDSKY, MD\*  
JOSEPHINE PRZEPIORA, MS, MT(ASCP)SM†  
SCOTT W. RIDDELL, PhD, MT‡  
DEANNA L. KISKA, PhD‡

\*Departments of Pediatrics  
†College of Health Professions  
‡Department of Pathology  
Upstate Medical University  
Syracuse, NY 13210  
lekurlandsky@yahoo.com

1. Johnston NW. The similarities and differences of epidemic cycles of chronic obstructive pulmonary disease and asthma exacerbations. *Proc Am Thorac Soc*. 2007; 4:591–596.
2. Wyse DM, Malloch D. Christmas tree allergy: mould and pollen studies. *Can Med Assoc J*. 1970;103:1272–1276.

3. Watson C, Rockwell W, Santilli J. Mold allergy and live Christmas trees. Paper presented at: American College of Allergy, Asthma & Immunology Annual Meeting; 2007; Dallas, Texas.
4. Iwen PC, Hinrichs SH, Rupp ME. Utilization of the internal transcribed spacer regions as molecular targets to detect and identify fungal pathogens. *Med Mycol*. 2002;40:87–109.
5. Pounder JI, Simmon KE, Barton CA, Hohmann SL, Brandt ME, Petti CA. Discovering potential pathogens among fungi identified as nonsporulating molds. *J Clin Microbiol*. 2007;45:568–571.
6. Pitkaranta MT, Meklin T, Hyvarinen A, et al. Analysis of fungal flora in indoor dust by ribosomal DNA sequence analysis, quantitative PCR, and culture. *Appl Environ Microbiol*. 2008;74:233–244.
7. Ciardo DE, Schar G, Altwegg M, Bottger EC, Bosshard PP. Identification of moulds in the diagnostic laboratory: an algorithm implementing molecular and phenotypic methods. *Diag Microbiol Infect Dis*. 2007;59:49–60.
8. Rosenbaum PF, Crawford JA, Anagnost SE, et al. Indoor airborne fungi and wheeze in the first year of life among a cohort of infants at risk for asthma. *J Expo Sci Environ Epidemiol*. 2009;20:503–515.

## ENTEROVIRUS-SPECIFIC IGG IN INTRAVENOUS IMMUNOGLOBULIN PREPARATIONS

Patients with humoral immunodeficiencies, such as common variable immunodeficiency and X-linked agammaglobulinemia, are at increased risk of developing enterovirus infections.<sup>1</sup> In an immunocompetent host, more than 90% of nonpolio enterovirus infections are asymptomatic or result only in a mild febrile illness. However, enterovirus infections in patients with humoral immunodeficiency often become chronic and more severe and may manifest as meningoencephalitis, hepatitis, dermatomyositis, arthritis, or myocarditis.<sup>2,3</sup>

Currently, no definitive therapy is available for severe enterovirus infections in immunodeficient patients, but different therapeutic approaches have been attempted. Pleconaril, an experimental antiviral drug initially studied for use against rhinovirus, showed some promise in the treatment of enteroviral infections, but its use was eventually discontinued because of safety concerns. Intravenous immunoglobulin (IVIG) is the main therapeutic modality in humoral immunodeficiencies and may play some role in prophylaxis against the development of enterovirus infections. Enterovirus-specific IgG antibodies in IVIG preparations are likely responsible for the prophylactic effects, and enterovirus antibody titers have been shown to increase after IVIG infusions.<sup>4</sup> However, enterovirus infections may still occur in patients receiving IVIG. In this scenario, replacement-dose IVIG, high-dose IVIG, and intraventricular and intrathecal immunoglobulin have been attempted, with mixed results and no formal studies.<sup>1,3,5</sup> One logical recommendation is that the IVIG preparations administered to immunodeficient patients with enterovirus infections should contain high quantities of enterovirus-specific IgG. Unfortunately, the amount of enterovirus-specific IgG in various IVIG products is not known or reported.

The goal of this study was to determine whether enterovirus-specific antibody is present in various commercially available IVIG preparations and, if present, quantify the amount and assess for differences among the IVIG products. To achieve this, 1-mL samples of 4 commercially available brands (2 with 2 distinct lots) of IVIG were obtained and converted to 5% solutions. Each sample was analyzed via enzyme-linked immunosorbent assay (ELISA) (Serion ELISA Classic ESR 133G Kit; Institut Virion/Serion GmbH, Wurzburg, Germany), which specifically detects enteroviral IgG.

**Disclosures:** Authors have nothing to disclose.

© 2011 American College of Allergy, Asthma & Immunology.

Published by Elsevier Inc. All rights reserved.

doi:10.1016/j.anai.2011.03.007